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09/806,462	03/22/2002	Koji Kigawa	084335/0134	4495

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EXAMINER	
STRZELECKA, TERESA E	

ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 07/22/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/806,462	KIGAWA ET AL.	
	Examiner	Art Unit	
	Teresa E Strzelecka	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 May 2004*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9,13 and 22-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9, 13, 22-24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. This office action is in response to an amendment filed May 12, 2004. Claims 1-21 were previously pending, with claims 10-12, 15, 16 and 18-21 withdrawn from consideration. Applicants amended claim 2, cancelled claims 10-12 and 14-21 and added new claims 22-24. Claims 1-9, 13 and 22-24 are pending and will be examined.

2. Applicants' amendments and claim cancellations overcame the following rejections: rejection of claims 1-9, 13, 14 and 17 under 35 U.S.C. 112, second paragraph; rejection of claim 17 under 35 U.S.C. 102(b) over Kigawa et al. All other rejections are maintained for reasons given in the "Response to Arguments" section below.

Response to Arguments

3. Applicant's arguments filed May 12, 2004 have been fully considered but they are not persuasive.

A) Regarding the rejection of claims 1, 2, 4-9 under 35 U.S.C. 102(b) as anticipated by Kigawa et al., Applicants argue the following:

a) Kigawa et al. provide ranges of concentrations of recombinase reaction components, and ranges do not anticipate specific concentrations,

b) the specific reaction mixture No. 23 from Table 1 on page 26, cited by the examiner as providing a specific example of reaction conditions which directly anticipate claim 1, because "the claims do not presume the use of GTPγS as the non-hydrolyzable nucleotide cofactor", the volume of the reaction mixture used by examiner was 9 μl, whereas it should have been 18 μl, since the first 9 μl was added to another solution, therefore the RecA concentration is two-fold lower, so the ration of GTPγS/RecA is 13.7, which is higher than 10.

c) using ratios claimed Applicants obtained unexpected results of improving reaction efficiency by reducing the amount of the non-hydrolyzable nucleotide cofactor in the reaction system.

Regarding a), even if the arguments in b) and c) were correct (they are not), since Kigawa et al. discloses ranges of working concentrations for the reaction components, it would involve routine, non-inventive experimentation to arrive at optimum results, see *In re Aller*, 105 USPQ 233 at 235:

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Regarding b) Applicants arguments are not persuasive for the following reasons:

1) claim 1 is drawn to any non-hydrolyzable cofactor, therefore they do, using Applicants terminology, "presume the use of GTPγS as the non-hydrolyzable nucleotide cofactor",

2) as to the specific example offered by reaction mixture No. 23, the use of a 9 μl volume for the reaction mixture (Example 2A(a)) is correct, since claim 1 is drawn to preparing a mixture comprising a single-stranded nucleic acid probe with RecA recombinase in the presence of non-hydrolyzable nucleotide cofactor, which are the three components present in the reaction mixture of Example 2A(a). Therefore, it is this very mixture at a volume of 9 μl which anticipates claim 1.

Regarding c), the unexpected results, Applicants presented them only for RecA, and the claims are drawn to any recombinase. Further, analysis of the results presented in Tables 1 and 2 does not really indicate the presence of unexpected results. First, the results of specificity determination were not presented for mixtures A and B at zero salt concentration. Secondly, the mixtures for which supposedly unexpected results were obtained and shown at zero salt (mixtures C, E, G, H, I, J, K and L) had both the ATPγS/nucleotides and ATPγS/RecA ratios varied, therefore

it is not clear whether lowering the ATPγS/RecA ratio is truly the factor solely responsible for the observed results. Finally, as can be seen in the results of reaction specificity for reaction mixtures A-K obtained with 100 mM NaCl, the differences in specificity are not dramatic, therefore, it seems that addition of 100 mM NaCl neutralizes differences arising from varying the ratios of ATPγS/nucleotides and ATPγS/RecA. Therefore, the unexpected results, if present at all, are certainly not visible at 100 mM NaCl, and would not apply to any reaction conditions.

Further, looking at the results of transformation specificity presented by Kigawa et al. in Table 2 on pages 26 and 27, it is clear that the ratio of co-factor to the nucleotide in the probe has an effect on the reaction specificity. For example, reactions 14-18 in Table 1 contained decreasing total amounts of the 275 bp probe and the same amount of RecA, 6 μg (the GTPγS/RecA ratio for this reaction would be 17/1), and the reaction specificity increased with decreasing amount of the probe, with reaction 18 having higher specificity than reaction 23 (page 27). Further, reaction 22 of Table 1, which differed from the cited reaction 23 in that it contained half the amount of RecA (the GTPγS/RecA ratio for this reaction would be 13.6/1) and half the amount of non-specific probe had higher reaction specificity than reaction 23. Therefore, Applicants' claims to obtaining unexpected results seem unfounded from the point of view of both the results presented in the specification and the results presented by Kigawa et al.

The rejection is maintained.

B) Regarding rejection of claim 3 under 35 U.S.C. 103(a) over Kigawa et al. and Sena et al. and rejection of claim 13 under 35 U.S.C. 103(a) over Kigawa et al. and Kigawa-2 et al., Applicants argue that since Kigawa et al. do not anticipate claim 1, these claims are not suggested by the combination of references.

The arguments regarding anticipation of claim 1 by Kigawa et al. were presented above.

The rejections are maintained.

New rejections will be presented for newly added claims 22-24.

Claim Rejections - 35 USC § 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claims 1, 2, 4-9 and 22 are rejected under 35 U.S.C. 102(b) as being anticipated by Kigawa et al. (WO 98/08975; cited in the previous office action), as evidenced by Sena et al. (U.S. Patent No. 5,670,316; cited in the previous office action).

Regarding claim 1, Kigawa et al. teach a method of preparing a RecA/single-stranded nucleic acid probe, the method comprising reacting a single-stranded nucleic acid probe sample containing a homologous probe with a RecA-like recombinase in the presence of a nonhydrolyzable nucleotide co-factor the number of molecules of which is one quarter or more of the number of molecules of nucleotide residues in the single-stranded nucleic acid probe and 10 times or less the number of molecules of the RecA-like recombinase (Kigawa et al. teach preparing a RecA-like recombinase/single-stranded DNA complex by mixing a RecA-like recombinase with the homologous nucleic acid probe in the presence of non-hydrolyzable nucleotide cofactor, GTPγS or ATPγS (page 15, lines 4-14; page 14, lines 15-28). Kigawa et al. teach that the reaction mix may contain 0.05-5 mM GTPγS, or 0.01-3 mM ATPγS or 0.3-3 mM ATPγS, 0.002-0.025 mM RecA protein, and 0.5-150 ng of homologous probe per reaction (page 17, lines 2-5). Kigawa et al. do not specifically teach that a number of molecules (= molar concentration) of GTPγS or ATPγS is 10-fold or less than the number of molecules of RecA recombinase, however, if a reaction mix contains

0.05 mM GTPγS and 0.025 mM of RecA recombinase, the number of co-factor molecules is twice the number of RecA molecules. If the reaction mix contains 0.01 mM ATPγS and 0.025 mM of RecA recombinase, the number of co-factor molecules is 0.4 times the number of RecA molecules, etc. Therefore, there are quite a few combinations of reaction conditions under which the number of co-factor molecules is 10-fold or less than the number of molecules of RecA recombinase.

Furthermore, Kigawa et al. teach a specific reaction mixture, No. 23 (Table 1, page 26), which contained 1 ng of 275 bp homologous probe, 500 ng of λ DNA fragments and 15 μg of RecA. The reaction volume was 9 μl, and contained 0.3 mM GTPγS (page 23, lines 7-17). Again, Kigawa et al. do not specifically teach that a number of molecules (= molar concentration) of GTPγS is 10-fold or less than the number of molecules of RecA recombinase and one quarter (= 25%) or more of the number of molecules of nucleotide residue in the nucleic acid probe. However, calculation of the molar concentrations of RecA and nucleic acid probe gives the following values for the molar concentration of these two components: RecA concentration of 0.044 mM (assuming molecular weight 37,842, as evidenced by Sena et al., col. 2, lines 42, 43), and concentration of the homologous 275 bp probe of 0.011 μM (assuming a molecular weight of one nucleotide of 330). Therefore, the molar ratio of GTPγS to RecA is 6.8, which is less than 10-fold, and the molar ratio of GTPγS to nucleotide residue in the nucleic acid probe is 27,000, which is more than 25% of the number of nucleotide residues in the homologous probe, and Kigawa et al. anticipate limitations of claim 1.)

Regarding claims 2 and 22, Kigawa et al. teach ATPγS and ADP. AlF_4 (page 14, lines 15-18).

Regarding claim 4, Kigawa et al. teach a mixture of homologous and heterologous probes (page 8, lines 16-23; page 16, lines 28-31; page 17, lines 1-5; page 23, lines 7-18).

Regarding claim 5, Kigawa et al. teach magnesium ion concentrations from 1-30 mM (page 16, line 31), or 2 mM (page 23, lines 13-18), anticipating the range of 0.5 to 2 mM.

Regarding claims 6 and 7, Kigawa et al. teach RecA from E. coli (page 9, line 14).

Regarding claim 8, Kigawa et al. teach RecA-like recombinase which has a label (page 15, lines 17, 18).

Regarding claim 9, Kigawa et al. teach a homologous probe which has a label or a ligand (page 12, lines 18-23).

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kigawa et al. (WO 98/08975; cited in the previous office action) and Sena et al. (U.S. Patent No. 5,670,316; cited in the previous office action).

A) Teachings of Kigawa et al. are discussed above. Kigawa et al. do not teach a homologous probe consisting of two at least two types of homologous probes that are sufficiently complementary to one another.

B) Sena et al. teach double-stranded probes for homologous recombination, the probes consisting of two sequences containing regions of complementary overlaps with each other, with a degree of complementarity between 70 and 100% (col. 3, lines 39-44; col. 12, lines 29-46), which is the degree of complementarity considered as substantial by Applicants (page 10, lines 35, 36; page 11, lines 1-10), therefore Sena et al. teach probes with substantially complementary overlap.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the double-stranded probes of Sena et al. in the method of Kigawa et al. The motivation to do so, provided by Kigawa et al., would have been that using double-stranded probes produced probe:target DNA complexes stable to deproteinization (col. 3, lines 25-30).

8. Claims 13 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kigawa et al. (WO 98/08975; cited in the previous office action) and Kigawa-2 et al. (EP 0 687 738 A1; cited in the previous office action).

A) Regarding claim 13, Kigawa et al. teach RecA-like recombinase labeled with a label or a ligand, but do not teach biotin or digoxigenin. Regarding claim 14, Kigawa et al. teach isolation of target DNA by capturing a complex of RecA/co-factor/probe labeled with biotin/target DNA with magnetic beads bound to streptavidin (page 17, lines 20-27).

B) Regarding claim 13, Kigawa-2 et al. teach RecA protein labeled with biotin or digoxigenin (col. 10, lines 1-15).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used labeled RecA protein of Kigawa-2 et al. in the method of Kigawa et al. The motivation to do so, provided by Kigawa-2 et al., would have been that labeling the protein provided a sensitive and simple method of detecting hybridization complexes (col. 4, lines 3-11).

9. Claims 23 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kigawa et al. (WO 98/08975; cited in the previous office action).

A) Regarding claims 23 and 24, Kigawa et al. teach that the reaction mix may contain 0.05-5 mM GTPγS, or 0.01-3 mM ATPγS or 0.3-3 mM ATPγS, 0.002-0.025 mM RecA protein, and 0.5-150 ng of homologous probe per reaction (page 17, lines 2-5). Kigawa et al. do not specifically teach that a number of molecules (= molar concentration) of GTPγS or ATPγS is 5-fold or less or 3-

fold or less than the number of molecules of RecA recombinase, however, if a reaction mix contains 0.05 mM GTPγS and 0.025 mM of RecA recombinase, the number of co-factor molecules is twice the number of RecA molecules. If the reaction mix contains 0.01 mM ATPγS and 0.025 mM of RecA recombinase, the number of co-factor molecules is 0.4 times the number of RecA molecules, etc. Therefore, there are quite a few combinations of reaction conditions under which the number of co-factor molecules is 10-fold or less than the number of molecules of RecA recombinase.

Furthermore, Kigawa et al. teach a specific reaction mixture, No. 23 (Table 1, page 26), which contained 1 ng of 275 bp homologous probe, 500 ng of λ DNA fragments and 15 μg of RecA. The reaction volume was 9 μl, and contained 0.3 mM GTPγS (page 23, lines 7-17). Again, Kigawa et al. do not specifically teach that a number of molecules (= molar concentration) of GTPγS is 10-fold or less than the number of molecules of RecA recombinase and one quarter (= 25%) or more of the number of molecules of nucleotide residue in the nucleic acid probe. However, calculation of the molar concentrations of RecA and nucleic acid probe gives the following values for the molar concentration of these two components: RecA concentration of 0.044 mM (assuming molecular weight 37,842, as evidenced by Sena et al., col. 2, lines 42, 43), and concentration of the homologous 275 bp probe of 0.011 μM (assuming a molecular weight of one nucleotide of 330). Therefore, the molar ratio of GTPγS to RecA is 6.8, which is less than 10-fold, and the molar ratio of GTPγS to nucleotide residue in the nucleic acid probe is 27,000, which is more than 25% of the number of nucleotide residues in the homologous probe, and Kigawa et al. anticipate limitations of claim 1.

Therefore, Kigawa et al. teach ranges of GTPγS/RecA ratios which are less than five-fold or three-fold, and a specific ratio which is 6.8/1.

Further, looking at the results of transformation specificity presented by Kigawa et al. in Table 2 on pages 26 and 27, it is clear that the ratio of co-factor to the nucleotide in the probe and to the RecA, as well as the amount of non-specif DNA present, can all be adjusted to obtain the desired reaction specificity. For example, reactions 14-18 in Table 1 contained decreasing total amounts of the 275 bp probe and the same amount of RecA, 6 μg (the GTPγS/RecA ratio for this reaction would be 17/1), and the reaction specificity increased with decreasing amount of the probe, with reaction 18 having higher specificity than reaction 23 (page 27). Further, reaction 22 of Table 1, which differed from the cited reaction 23 in that it contained half the amount of RecA (the GTPγS/RecA ratio for this reaction would be 13.6/1) and half the amount of non-specific probe had higher reaction specificity than reaction 23.

Thus, an ordinary practitioner would have recognized that the optimizable variables of the concentrations of could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific concentrations of components was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have adjusted the concentrations of non-hydrolyzable cofactor, recombinase and probe

in the reaction mixture, as indicated by Kigawa et al. The motivation to do so would have been to optimize the reaction specificity and efficiency.

10. No claims are allowed.

Conclusion

11. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Teresa E Strzelecka whose telephone number is (571) 272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

TS
July 12, 2004

JEFFREY FREDMAN
PRIMARY EXAMINER
7/16/04